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RELATIVE TOXICITY OF HYDRAZINE PROPELLANTS TO A SOIL BACTERIUM

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FOR THE COMMANDER

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The applicability of bacterial systems as an indicator of environmental toxicity was determined by observation of the effect of selected missile propellants on the growth dynamics of a soil microorganism. A soil isolate capable of rapid growth in a mineral salts medium was exposed to various concentrations of the propellants hydrazine (Hz), unsymmetrical dimethylhydrazine (UDMH), and monomethylhydrazine (MMH). Growth of the exposed cultures was followed turbid-

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Block 20. Abstract, cont'd

imetrically and compared to unexposed cells. Ten experiments were conducted indicating the order of toxicity to be Hz > MMH > UDMH. The bacterial response was a delay in the onset of log growth, with as little as 0.05 ppm Hz exerting on inhibitory effect. Responses were dose dependent and suggested a two-phased mechanism consisting probably of a bacteriocidal and a bacteriostatic component.

PREFACE

This study was conducted in the Toxic Hazards Division, Environmental Quality Branch, Air Force Aerospace Medical Research Laboratory. This research was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials;" Task 04, Work Unit 19, from April 1975 to December 1976.

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INTRODUCTION

In an age of increasing dependence on chemical compounds to expand the range and efficiency of human activity, new molecular species are introduced each year into the industrial, commerical, and residential areas, in increasing numbers. Estimations of 500 to 1,000 new substances added each year to a list of over 50,000 indicate the extent of this dependence and suggest the potential toxicologic impact to man and the environment this may represent. Certainly those materials that are used either in exceedingly large quantities or ubiquitously pose a potential hazard. The nature and ramification of the hazard must be determined by acceptable toxicology procedures and include the necessary toxicity data as well as information on environmental stability and fate, biotransformation, bioaccumulation, fire and explosive hazards, and nuisance factors. These considerations must also include all phases in the manufacture, transportation, distribution, use, waste production, and decontamination and disposal of the compound. Added to this burden is the requirement to insure that long-term effects on man or the environment do not occur. These include mutagenic and carcinogenic effects on man and both transient and permanent ecologic disruptions to the environment.

Biological systems of varying degrees of complexity are selected as laboratory models for the investigation of environmental toxicity based on the interest of the investigator, the kinds of data required, relationship to the actual target, and cost, time, and handling constraints. To reduce the number of compounds to be evaluated by more rigorous and costly mammalian toxicology methods to a level comensurate with available resources, significant emphasis is being given to in vitro or short term toxicity tests. These tests represent a diversity of biological systems including bacteria, yeasts, plant and animal tissue culture, insects and amphibians.

Compounds of particular interest to the Air Force are the hydrazines since they are used in large quantities as missile propellants and are quite toxic. The chemistry of these substances has been amply described (Malone, 1970; Audrieth and Ogg, 1951; Slonim and Gisclard, 1976; Slonim, 1975; Gormley and Ford, 1973; Luker, 1976; Harshman, 1957). Clark et al. (1968) presented an extensive review of the toxicologic and pharmocologic aspects of hydrazine fuels. More recently, Back et al. (1978) described the current knowledge of the occupational hazards and included a discussion of environmental implications and the use of non-mammalian systems for toxicologic and environmental investigations. Earlier field studies on the environmental impact of hydrazine fuels were described by Kennebeck et al. (1963). Nore recent investigations have been concerned with effects on various aquatic species (Klein, 1978; Sherfig et al., 1978, 1977; and Fisher et al., 1978). The mutagenicity of these compounds in in vitro systems has been considered by Brunsick and Matheson (1976a, 1976b).

To ascertain the utility of bacterial systems to provide quantitative data on the toxicity of environmental contaminants, we determined the effect

of hydrazine (Hz), monomethylhydrazine (MMH), 1,1-dimethylhydrazine (UDMH), and Aerozine-50 (A-50 a 50/50 mixture by weight of Hz and UDMH) on the growth kinetics of a soil bacterium.

MATERIALS AND METHODS

Organism: The selection of the test bacterial strain was based on the following requirements: a constituent of a representative soil community; capable of aerobic growth in a glucose-mineral salts medium; mean generation time of 30-60 minutes; and moderately sensitive to hydrazine fuels. Several grams of soil were obtained from the top five (5) cm non-amended, non-agriculture soil and incubated with shaking at room temperature for 48 hours in a glucose - mineral salts broth. Aliquots of this mixed culture were plated on glucose-mineral salts agar, incubated for 36 hours at room temperature, and representative colonies, picked and streaked on the same medium. Numerous strains were evaluated by the above criteria and one, designated D-31, was selected as the test organism and tentatively identified as Paracoccus denitrificans. The culture was maintained either in the lyophilized state or on slants of Tryptic Soy Broth (Difco) plus 1.5% Bacto-Agar (Difco).

Experimental Conditions: The medium used throughout this study was a glucose-mineral salts broth prepared with glass distilled water and of the following composition per liter:

KH_2PO_4	-	0.950g
K_2HPO_4	-	2.440g
$(NH_4)_2SO_4$	-	1.000g
$MgSO_4 \cdot 7H_2O$	-	0.200g
$FeCl_3 \cdot 6H_2O$	-	0.001g
$CaCl_2$	-	0.015g
Glucose	-	2.000g

The above glucose concentration was selected since it is rate limiting and will be utilized in continuous culture studies with this organism. The 0.2 percent level was determined to be most appropriate for both batch and steady state experiments by growth rate studies. The salts were reagent grade obtained from MCB; glucose was purchased from Nutritional Biochemicals. One hundred mℓ aliquots of the mineral salts medium (SMS) were added to 500 mℓ Nephelo Culture flasks (Bellco #2574-19135), the cleanout screw-caps replaced with rubber serum stoppers, and the flasks autoclaved for 15 minutes and cooled. Filter-sterilized glucose was added to the flasks with a syringe through the serum stopper and the

flasks incubated for several days at room temperature to insure sterility. The desired concentration of the four hydrazine fuels in ppm by volume was obtained by adding the required volume of fuel or an aqueous dilution of the fuel with a microliter syringe. When higher concentrations of fuel were used, sterile HCl was added to the flasks to reduce the pH to the original level of 7.1. Ten different experiments were performed in which concentration, source of propellant, duration, and inoculum size were varied. Sources of the hydrazine fuels used included: hydrazine - MCB, Lot 4F24; MMH - MCB, Lots 20 and 23 and Eastman Lot #711-4; UDMH: redistilled samples from unidentified pooled sources; and A-50 - formulated from mixtures of Hz and UDMH from above samples. Stability of the hydrazine fuels in glass distilled water and SMS was determined as described by Watt and Crisp (1952), Hoover et al., 1964, and Smith and Karty (1968). Subsequent to the addition of the fuels, the flasks were inoculated with 0.1 mℓ of a 16 hr glucose - SMS broth culture with a tuberculin syringe through the serum stopper. Inoculum concentration was in the range of 10-25 x 10^8 cfu/mℓ, equivalent to a turbidity of approximately 35-25% transmittance at 570 nm as determined with a Coleman Jr. Model 6D spectrophotometer. The flasks prepared in duplicate or triplicate were incubated on a reciprocating shaker (Eberback 6,000 fitted with a large carrier and flask holders) at an oscillation rate of 100/min at room temperature (21±1°C). Cell mass was determined turbidimetrically usually at intervals of 2 hr initially and at more extended intervals towards termination of the experiment for periods up to 283 hr. The rate limiting concentration of glucose was determined in a similar manner.

To ascertain if the effect of the hydrazines was bacteriocidal, several brief experiments were conducted at the lowest concentrations of the fuels that appeared to cause complete growth inhibition. Nephelo flasks were prepared as described, and at selected time intervals, 10 µℓ aliquots were removed aseptically and the presence of viable cells determined either qualitatively by inoculation of glucose - SMS broth and TS broth or quantitatively by plotting 0.1 mℓ dilutions on Plate Count Agar using the spread plate technique.

RESULTS

Rate-limiting glucose concentration - The selection of 2g/liter as the rate of limiting concentration of glucose was based on the growth response data shown in Figure 1. These data summarize several experiments in which glucose concentration, inoculum size, and experiment duration were varied. The 2.0g/liter (4.40 mM) level was chosen since it produced a desirable culture density of ~ 20 x 10^8 cfu/mℓ at maximum growth which was obtained at approximately 16 hr incubation at room temperature. This amount of growth was readily determined turbidimetrically while providing sufficient cell mass for both acute (batch) and chronic (continuous) culture studies. In addition, acid formation from this amount of glucose could be monitored in subsequent chemostat studies as a useful index of culture activity.

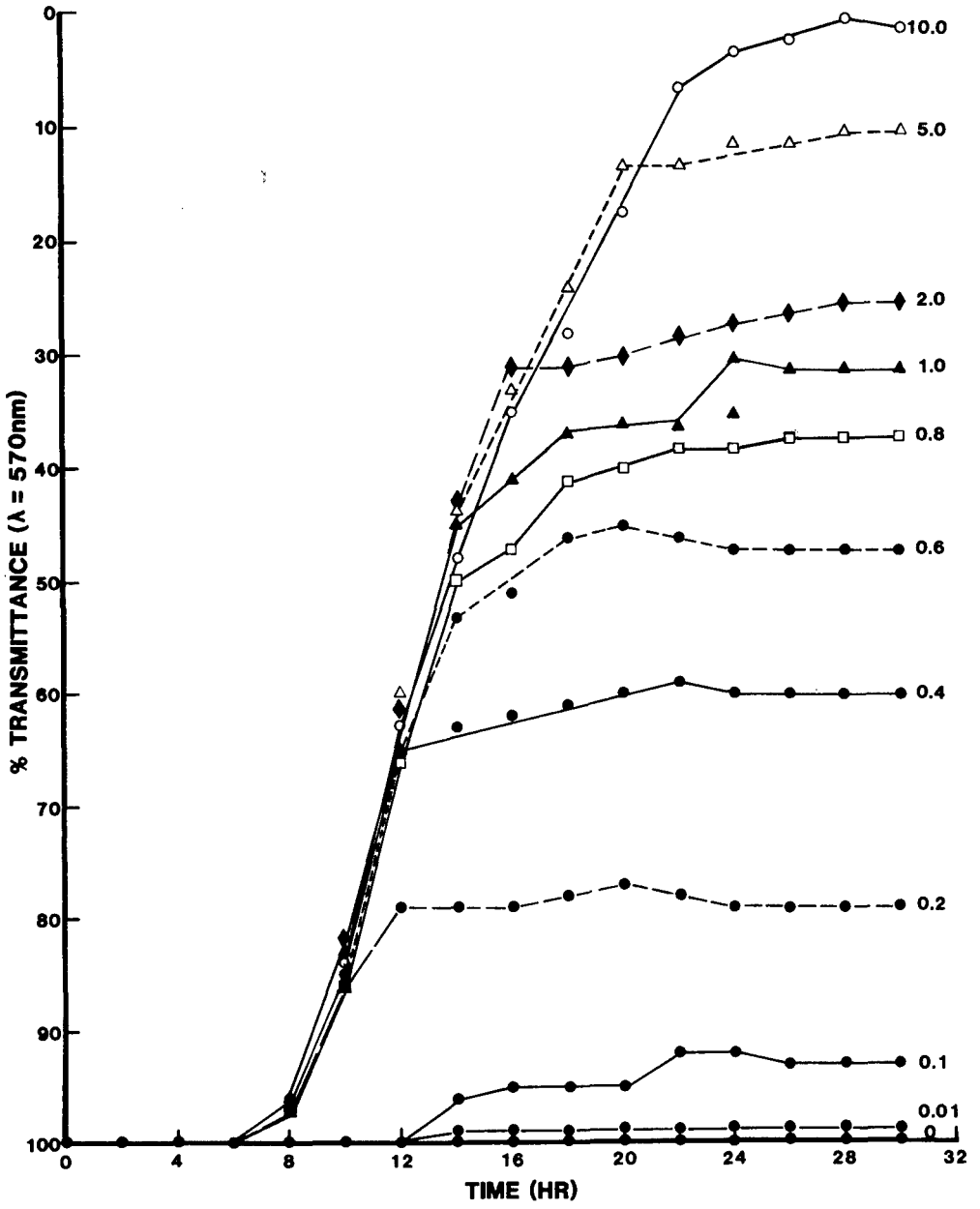

Figure 1. Effect of glucose concentration on the growth of D-31. Turbidity is expressed as % transmittance rather than optical density to expand curve relationships. Numbers refer to glucose concentration in g/L.

Fuel Stability - Since exposure of microbial cells or tissue culture to a toxic substance is analagous to an environmental exposure in that contact occurs from the environment medium (air or water) over some time period, stability of the compound under the conditions of exposure must be determined. The effect of the formulation of glucose-SMS medium on hydrazine stability at room temperature is shown in Figure 2. In addition to the compounds listed previously, this formulation contained a number of metal salts including copper, zinc, cobalt, molybdenum, and manganese in trace quantities. With the exception of 1 ppm, all concentrations of

hydrazine showed a decrease over the 7 day period, the 24.2 ppm level reduced by approximately 58%. These metal salts were eliminated subsequently from the medium composition, resulting in significant increase in the growth rate and final yield of D-31. However, no change in hydrazine stability was observed, indicated in Figure 3, as reported by other investigators (Scherfig et al., 1978, 1977, 1976). In general, these workers found that an increase in ionic and organic content appeared to increase the rate of hydrazine propellant decomposition. Hoover et al. (1964) listed the following factors in the decomposition of Hz and UDMH in aqueous solutions: a) trace amounts of copper or other ions; b) dissolved oxygen; c) decomposition rate is pH and temperature dependent; d) decomposition rate is influenced by propellant concentration. Scherfig et al. (1978, 1977) described propellant stability in deionized water (degradation less than 5% after 21 days) and the influence of medium composition, particularly trace metals on decomposition rates. Slonim and Gisclard (1976) presented related data concerning the effect of environmental water source, water hardness, and fish excretions on Hz degradation. Figures 2 and 3 show that the addition of trace metals to the basic glucose-SMS medium does not increase decomposition. Thus the basic medium composition accounts for all the observed fuel degradation in these studies. In addition to the propellant breakdown attributable to the medium constituents, some decomposition or at least reduction in concentration in the aqueous environment may occur due to reaction with bacterial metabolic products or adsorption by the cells. This will be investigated in a subsequent study.

Propellant Effects on D-31 Growth - Each of the hydrazine propellants was studied in numerous independent experiments in which source of propellants, inoculum size, agitation rate, etc. were varied. As anticipated, inoculum concentration did influence the time course of the measured effects since the number of propellant molecules available for reaction is determined by the initial concentration of propellant and the cell density (both viable and non-viable) as well as medium reduced degradation and system passivation influenced by the surface area volume ratio in the growth chamber. The results shown in Figures 4-7 represent individual experiments with Hz, MMH, UDMH, and all three propellants plus A-50, respectively, in which experimental conditions including inoculum concentration were constant. To obviate any positional effects, the Nephelo flasks were rotated randomly on the shake table after each reading.

The use of statistical analysis to determine significance under the conditions of these experiments was considered, however, since the curves presented resulted from turbidimetric data which were obtained only in duplicate, statistical comparison of curves to indicate biological significance was not thought to be meaningful. Repeated experiments in which inoculum concentration and physiological status, propellant concentration, and incubation temperature could not be duplicated exactly did provide reproducible trends. The relatively consistent growth dynamics of D-31 control cultures allow a comparison to be made by direct examination of the growth cultures.

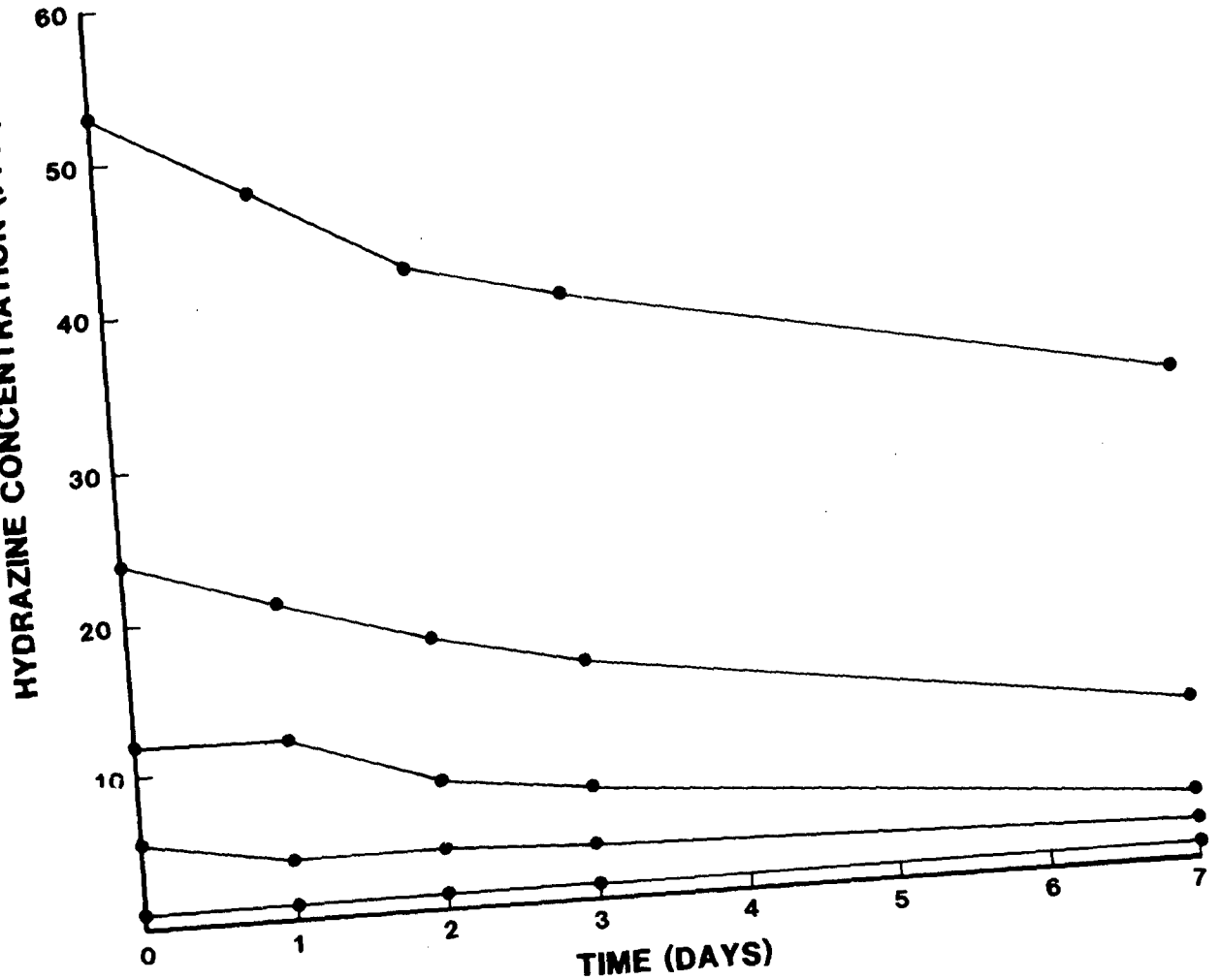

Figure 2. Hydrazine decomposition in modified glucose-SMS medium at room temperature.

Characteristically, D-31 when inoculated in SMS broth containing 4.4 mM glucose at 21 ± 1C attained maximal growth in approximately 16 hours. This represents a cell density of ca. 20 x 10^8 cfu/mℓ and is equivalent to a turbidity of approximately 30%T at 570 nm. Deviations from these values were indicative of interference with the growth activities of the culture. Although hydrazine exposure resulted in a delay in the onset of the log growth phase, the final growth yield (cell density) was essentially identical to that of the control (Figure 4). The smallest concentration shown (0.05 ppm) induced a delay of about 45 min while 10 ppm delayed initiation of log phase for 48.5 hours. The higher concentrations of Hz (50, 100, and 500 ppm) resulted in no visible growth for at least 72 hours at which time the experiment was terminated.

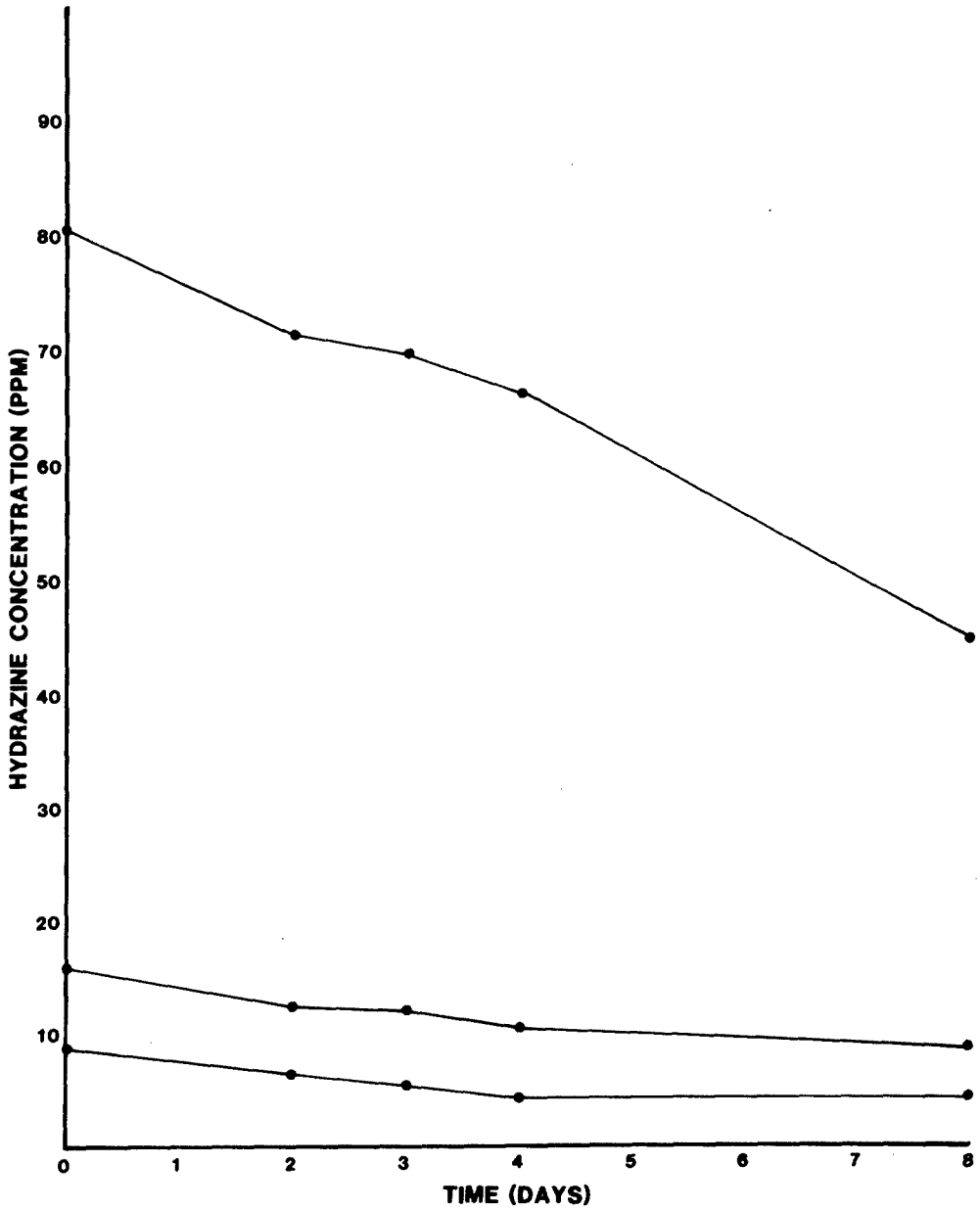

Figure 3. Hydrazine decomposition in glucose-SMS medium at room temperature.

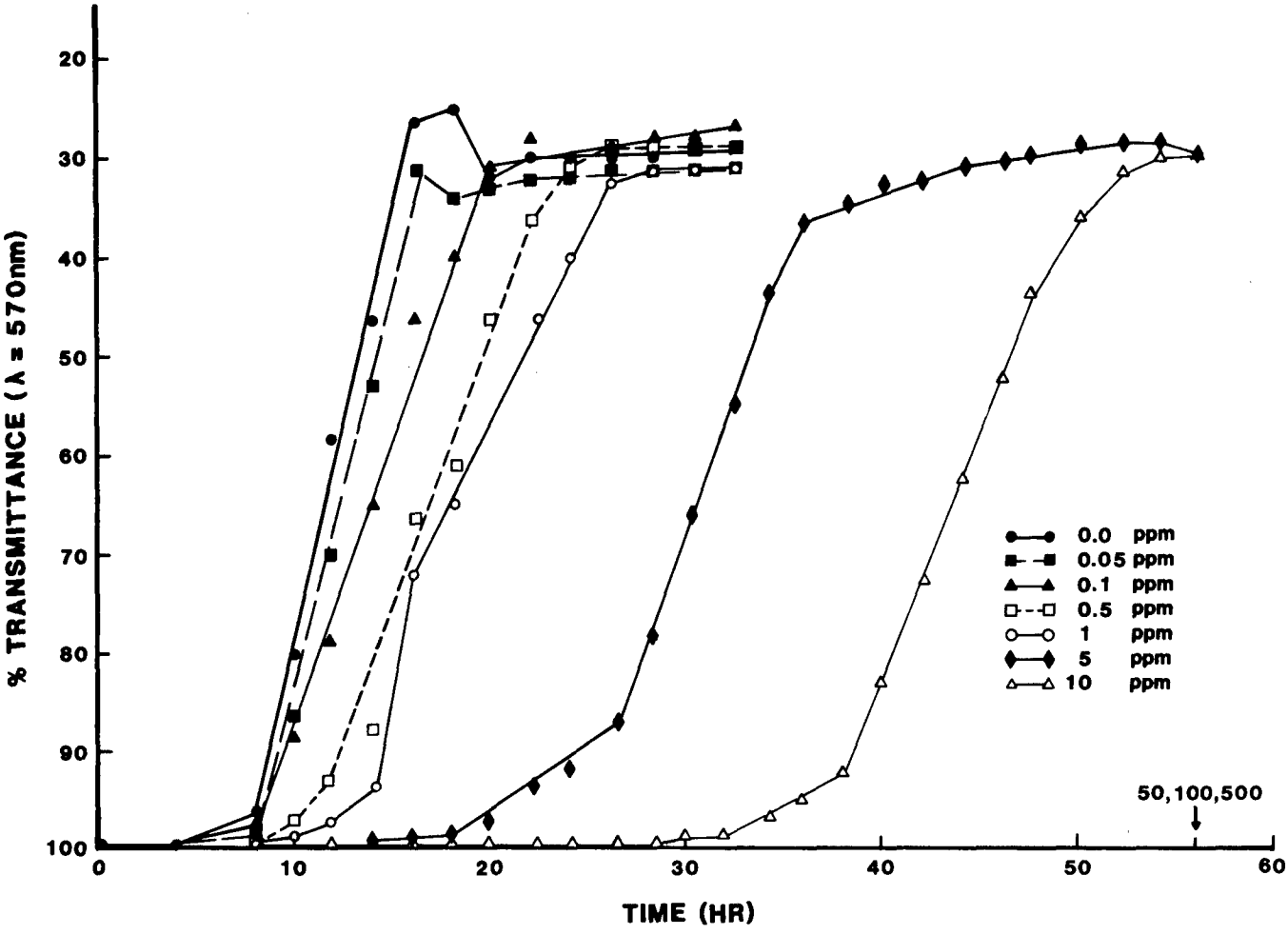

Figure 4. Effect of various concentrations of monomethylhydrazine on the growth of D-31.

Exposure of D-31 to MMH manifested results similar to Hz in that onset of log growth was delayed while maximal growth was comparable to control values as shown in Figure 5, although the higher MMH concentrations (10 and 50 ppm) appeared to result in a somewhat decreased growth level. Since a lower final growth was observed in all of the experiments conducted with higher concentrations of MMH (approximately 6%T less than the control at 10 ppm and as much as 14%T at 50 ppm as shown in Figure 7), these differences are considered to be significant. In all experiments in which D-31 was exposed to 100 ppm MMH, growth did not occur (up to 283 hr as shown in Figure 7) and in one study that included qualitative determination

of viability, no growth occurred upon transfer to fresh medium after 72 hours of exposure, indicating death of the culture.

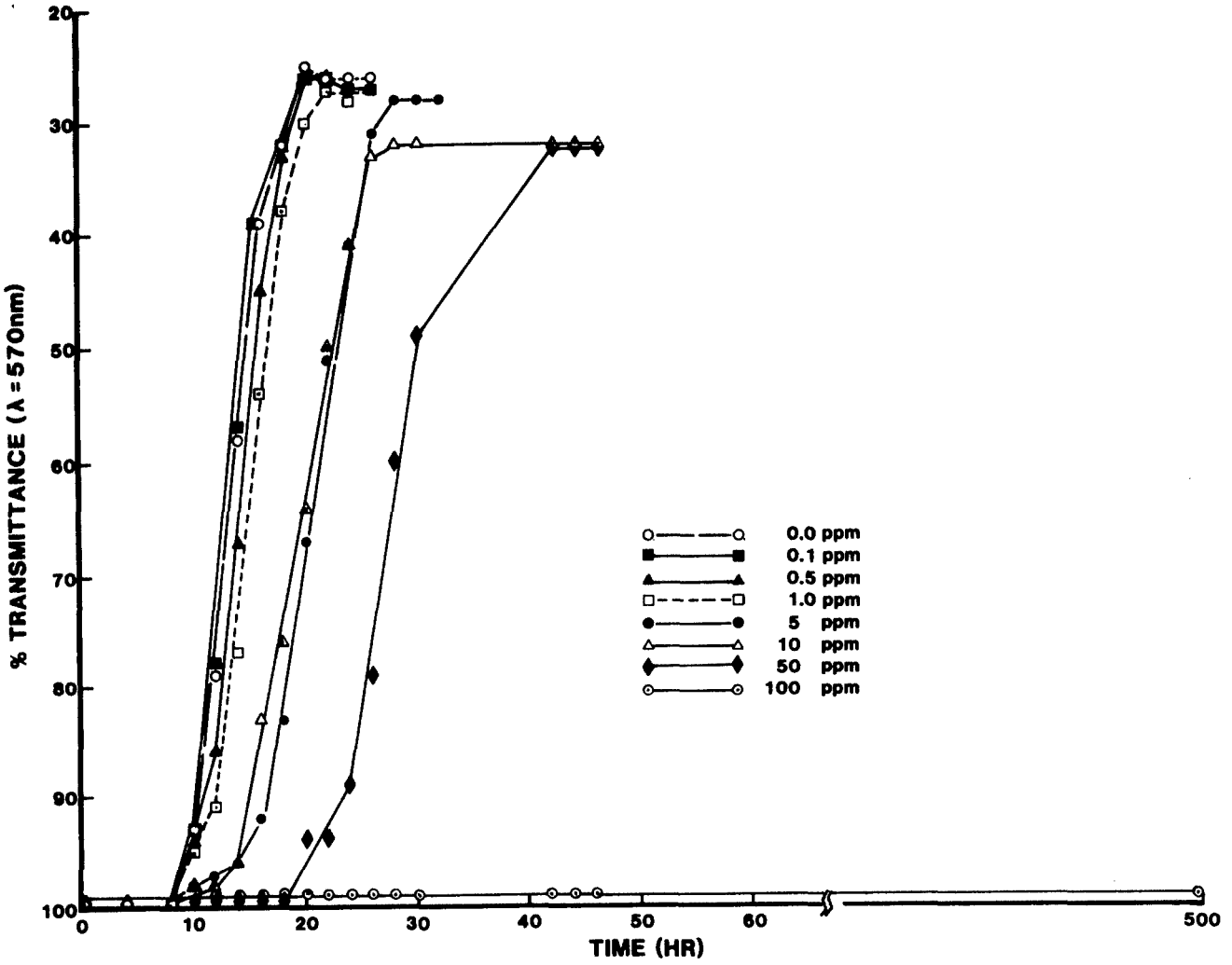

Figure 5. Effect of various concentrations of hydrazine on the growth of D-31.

The response of D-31 to a range of concentrations of UDMH is presented in Figure 6. These curves are representative of several experiments in which concentrations from 0.01 to 1,000 ppm were used. In a range finding experiment in which UDMH varied from 0.01 to 100 ppm and the inoculum was at a high concentration of approximately 27 x 10^8 cfu/mℓ (21.5%T), most of the growth responses were identical to the control, i.e. log phase was initiated at about 9 hr and maximal growth was obtained after 12 hr incubation, the final yield occurring at 27 ± 2%T. The 10 and 50 ppm cultures did show an increased lag of several hours while the growth curve of 100

ppm UDMH cultures was the same as the control. In all of the UDMH experiments, as represented in Figures 6 and 7, one concentration produced less inhibition than that of lower concentrations. In Figure 6, the response of 50, 100, and 200 ppm exposures resulted in growth inhibition as indicated by the increase (over the control) in the time for onset growth by 5 to 7 hr. Figure 7 shows a similar effect, 100 ppm indicates a delay in log growth of 93 hr whereas 1,000 ppm causes a delay of only 24 hours. Since

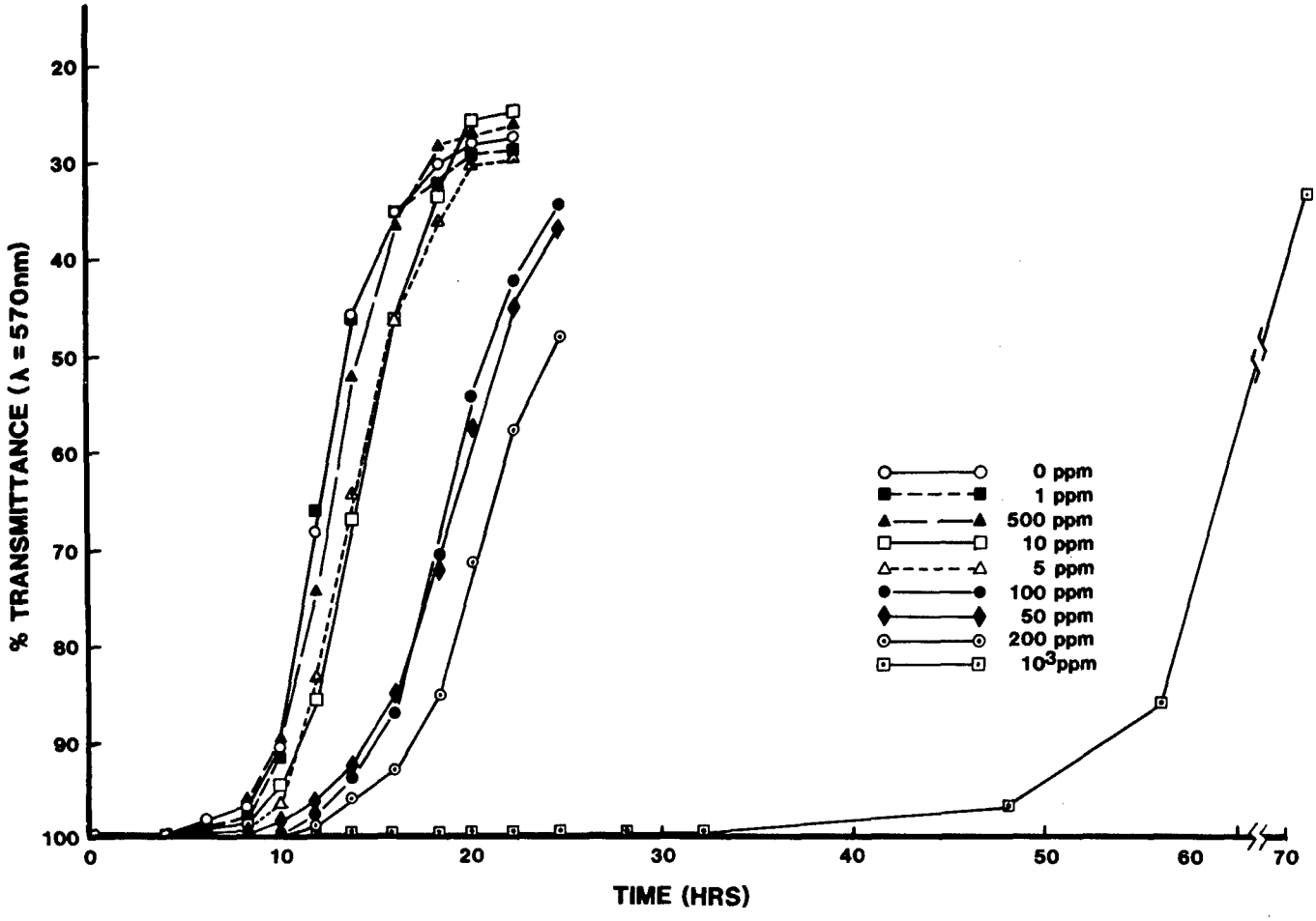

Figure 6. Effect of various concentrations of 1,1-dimethylhydrazine on the growth of D-31.

the degradation products of UDMH are qualitatively and quantitatively dependent upon the initial concentration of the propellant (Sikka et al., 1979) as well as pH, degree of oxygenation, and catalytic effects, these deviations from anticipated inhibitory activity at various exposure levels may be due to such differences in formulation of decomposition products.

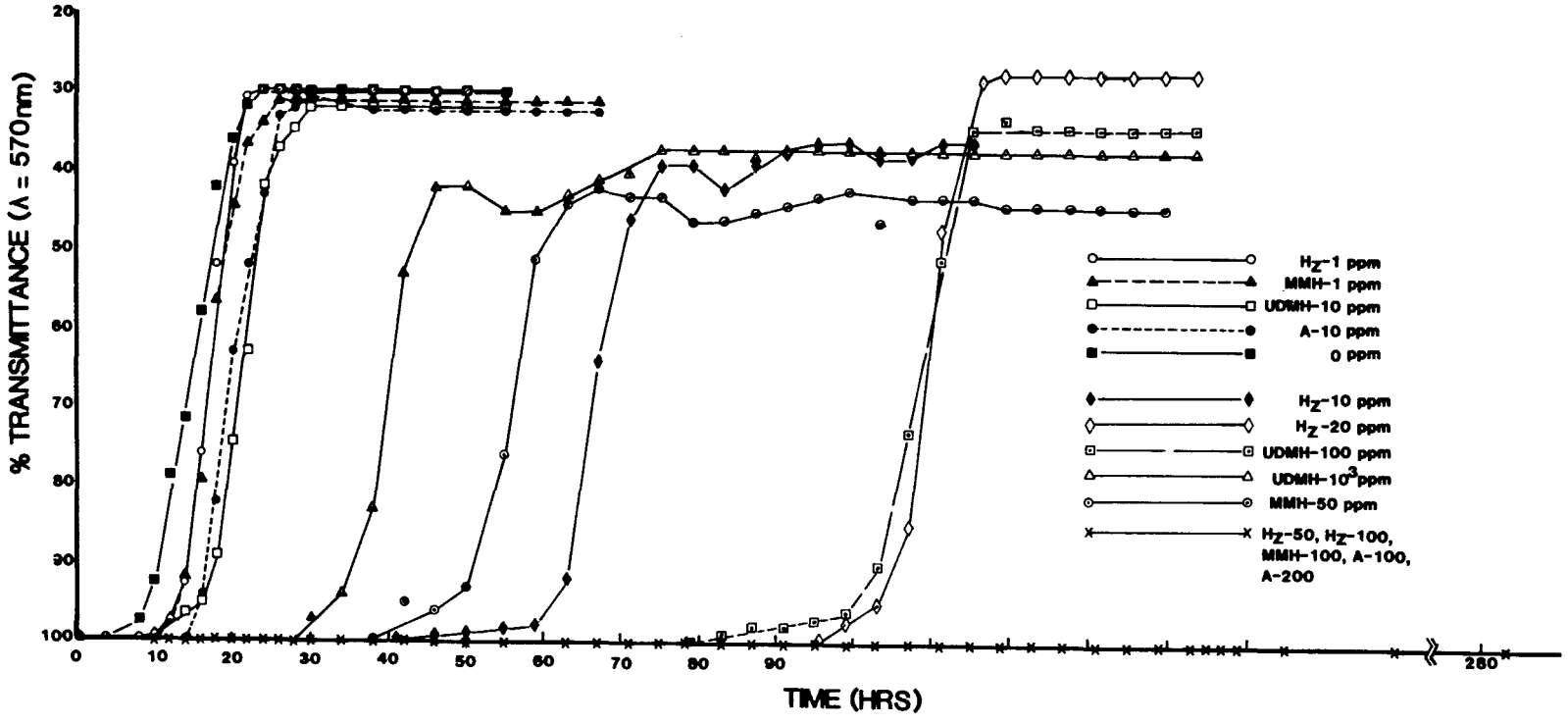

Figure 7. Effect of various concentrations of hydrazine, monomethylhydrazine, 1,1-dimethylhydrazine, and Aerozine-50 on the growth of D-31.

The major differences, other than noted previously, between UDMH and propellant effects previously described, was the lack of complete inhibition or killing at the highest concentration studied. An additional difference observed during some of the UDMH experiments was the development of a yellow color in the medium. Appearance of this color usually resulted in significantly delayed or no growth in the Nephelo flask and suggested a breakdown product of UDMH that possessed greater toxicity for D-31 than the parent compound. Sikka (1977) has observed a similar violet or yellow degradation product(s) that was formed in basic, aqueous solutions in the presence of trace quantities of copper ions. The colored compound derived from oxygenation of UDMH absorbed in the UV region at 326 nm and upon acidification the absorption shifted reversibly to 356 nm. A variety of substances resulting from the autooxidation of UDMH has been identified including dimethylnitrosoamine, diazomethane, nitrous oxide, methane, carbon dioxide, formaldehyde, ammonia, and dimethylamine (Urry et al., 1965 by Sikka, 1977). Presence of the first three compounds may have contributed to the color formation and bacterial inhibition in this study. Since the color did not occur with regularity it is assumed to have resulted from some contamination in the medium or flask appearing occasionally and exerting a catalytic effect on UDMH decomposition.

A summary experiment was conducted in which D-31 was exposed to selected concentrations of Hz, MMH, UDMH, and A-50 under identical conditions. The data obtained from this experiment shown in Figure 7 indicate:

1) 1 ppm Hz, 1 ppm MMH, 10 ppm UDMH, and 10 ppm A-50 caused a delay in onset of log growth (4-7 hr) but had no effect on final cell yield.

2) 50 and 100 ppm Hz, 10 ppm MMH, and 100 and 200 ppm A-50 completely inhibited growth for at least 283 hr.

3) 10 ppm Hz, 50 ppm MMH, and 100 and 1,000 ppm UDMH significantly delayed log growth (>30 to >100 hr) and resulted in a lower cell concentration at maximal growth.

4) 20 ppm Hz delayed log growth for approximately 105 hr and resulted in a small but significant increase in final growth level (27%T vs 30%T for the control).

The observed inhibitory effects of A-50 can be attributed to Hz since UDMH is considerably less effective than Hz. The concentrations of Hz to which D-31 was exposed in the A-50 mixture were 5, 50, and 100 ppm. The observed effects on the growth curve are thus as expected from such exposures.

Although deviation from the normal (control) growth patterns can be determined by comparison of curves resulting from exposure to various concentrations of propellant, more definitive information can be obtained by determining dose-response relationships as presented in Figure 8. These

curves were derived from the data shown in Figures 4-6 by plotting the difference in time to reach 65%T from the control versus the propellant concentration. A cell density of 65%T was selected since it approximates the inflection point of the curves and therefore represents a similar point in the growth dynamics of the individual cultures. Figure 8 clearly indicates the relative toxicity of the hydrazine propellants to D-31, Hz exerting the greatest effect and UDMH the least.

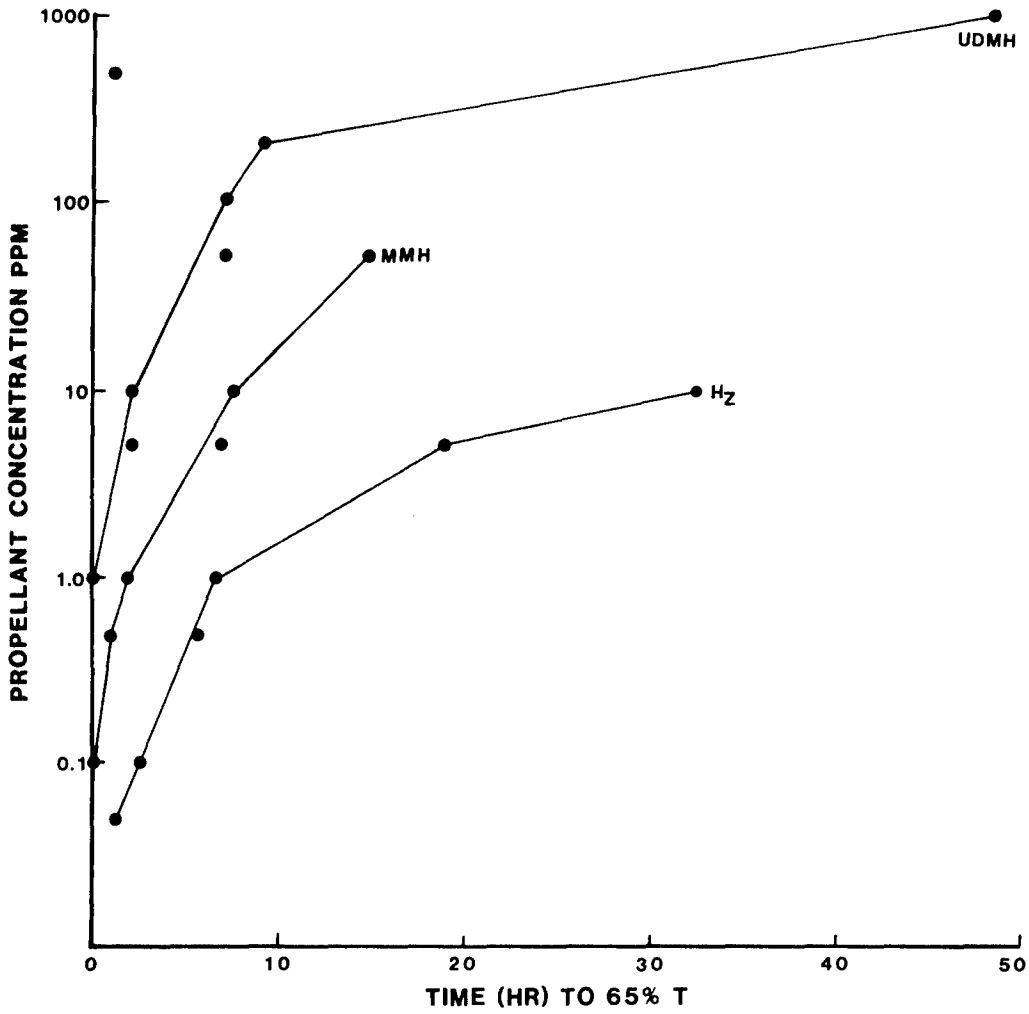

Figure 8. Effect of hydrazine, monomethylhydrazine and 1,1-dimethylhydrazine on the growth kinetics of D-31. Curves represent the growth rat rate at various propellant concentrations as indicated by length of growth period required to reach a cell concentration equivalent to 65%T corrected for control (unexposed) cultures.

Resistance to propellant inhibition: Several cursory experiments were conducted to ascertain the nature of the growth inhibition induced by Hz. The results of four of these quantitative studies, shown in Table 1, suggest that both bacteriocidal and bacteriostatic mechanisms are operative. This is indicated by the decrease in viable count over a protracted period followed by an eventual outgrowth of the culture after 246 hr in one experiment (Table 1D).

DISCUSSION

Before an evaluation of the data obtained from this series of experiments is performed, consideration should be given to the relationship of these data to actual biological events. There is a source of error inherent in turbidimetric data obtained with a transmission spectrophotometer. A direct relationship between cell concentration and turbidity (determined as % transmittance or optical density) is essentially impossible to obtain due to deviations from Beer's Law and to absorbance of the light energy of the selected wavelength. A more reliable and sensitive method for such measurements is nephelometry wherein light scattering is utilized as distinct from transmittance. However, the values of %T when plotted do provide reprensentative and reproducible information concerning the growth kinetics of bacterial cultures. Due to the lack of sensitivity of the method to indicate accurately cell concentration in terms of viable count (cfu/mℓ) and the inability to discern changes in total cell numbers at low concentrations, turbidimetric growth curves must be interpreted with caution. In particular, the time for the culture to attain a level of growth that is observable with the spectrophotometer could be construed as lag growth, i.e., no increase in cell concentration. In these experiments, this was probably not the case since the inoculum consisted of cells in the log growth phase (usually a 16 hr culture which for D-31 in glucose-SMS is at the end of the active growth period). Routinely 0.1 mℓ of a culture of approximately 10×10^8 cfu/mℓ was inoculated into 100 mℓ of medium resulting in a concentration of 1×10^6 cfu/mℓ. This is a sufficient number of cells to negate the effect of low level inocula that can result in lag rather than log growth from log phase cultures. This inhibition of log phase may be the result of trace contamination or insufficient concentrations of CO_2 necessary for growth (Davis et al., 1973). In some instances, log cells obtained from a minimal medium and inoculated into the same medium can revert to the lag phase. For these experiments, the delay observed in attaining visible growth will be construed as lag growth and any increase in that delay compared to the control is interpreted as a lengthening of the lag period and therefore is interference with the growth process. Subsequent experiments in which viable cell counts will be obtained will elucidate the actual bacterial growth activities.

The experiments reported permit several conclusions regarding bacterial growth response data as quantitative indicators of environmental toxicity:

(1) The most significant result of exposure, aside from death, was the lengthening of the lag period, in the extent of this period being related to pollutant concentration.

(2) When log growth occurred, the growth rate (μ, the specific growth

rate constant) was the same irrespective of propellant (or control) concentrations, i.e., the slopes of all the curves were the same for all concentrations of all propellants.

(3) At lower concentrations, final yields, i.e., viable count or turbidity, were the same; at higher concentrations, final yields less than the controls were observed.

Preliminary data have indicated that the increase in the lag phase is attributable to an initial killing effect followed by a process that is bacteriostatic in effect. This may be due to an interference with a synthetic pathway resulting in depletion of an essential metabolite, inhibition of energy transfer or transport mechanisms, etc. Ultimate reversal of this inhibition, whatever the basic mechanism may be due either to accumulation of the end product of the inhibited synthetic pathway or degradation of the propellant (inhibitor) to an ineffective level. Obviously other mechanisms may be involved; however, the data presented in Figure 8 are suggestive of dual mechanisms, since the derived curves appear to have two slopes. Definitive studies concerning the death rate in endogenously respiring and actively growing cultures will allow a better understanding of these phenomena.

That bacterial growth inhibition is a useful indicator of environmental toxicity can be appreciated by examination of the data compiled in Table 2. The experiments listed utilized a variety of aquatic species and indicated an effective concentration of the hydrazine propellants in approximately the same range. The bacterial response occurred over a range of <1 to 10 ppm with more significant effects at higher concentrations. Since bacterial data are in fact population responses rather than essentially individual organism (though replicated) responses, the results are not directly comparable. The tremendous amount of surface area presented by a bacterial culture, as discussed previously, is certainly an influence on the degree of toxicity expected. In addition, the duration of the experiment, medium composition, and influence of the waste products of the test species must be considered in terms of the degradation rate of the pollutant under study. In subsequent studies, measurement of the propellant concentration will be accomplished during the experiment period. Species sensitivity to a given toxic substance is also attributable to differences in detoxification mechanisms, both qualitative and quantitative, and the route of entry. These differences in the toxicity of hydrazine propellants to mammals are well documented (Clark et al., 1968). Since bacteria as a group exhibit a wide range of cellular composition and catabolic activity, the selection of one species is limiting. One means to reduce this limination and provide a somewhat more realistic test system is the use of mixed cultures. Selection of compatible strains capable of growing under the required conditions should be attempted.

The application of bacterial growth response (determined turbidimetrically) as an indicator of potential environmental toxicity is a useful addition to other screening procedures. It is a rapid and inexpensive procedure that does not require highly trained personnel and is suitably flexible to address a wide variety of environmental problems.

Table 1. Effect of 50 ppm hydrazine on survival of D-31 in glucose-SMS medium. Data represented results from four individual experiments.

A

Time-Hr	cfu x 10^5/mℓ Control	50 ppm Hz
0	25	30
1	TNTC	9.6
4	TNTC	7.4
24	$>10^8$	5.3
48	--	2.1
72	--	4.2

TNTC: Too numerous to count at dilution used.
-- Not counted.

C

Time-Hr	cfu x 10^5/mℓ Control	50 ppm Hz
0	23.2	29.6
1	6.5	14.2
4	--	7.5
24	$>10^8$	6.2
48	--	2.1
72	--	5.6
96	--	12.0
104	--	6.1

-- Not counted.
No visible growth in Hz up to 123 hours.

B

Time-Hr	cfu x 10^5/mℓ Control	50 ppm Hz
0	10.0	11.0
1	7.8	10.3
4	6.4	9.3
24	$>10^8$	5.8

No visible growth in Hz up to 186 hours.

D

Time-Hr	cfu x 10^5/mℓ Control	50 ppm Hz
0	8.2	12.8
1	12.7	12.5
4	11.6	7.6
24	$>10^8$	$<10^4$
48	--	$<10^4$
72	--	$<10^4$
168	--	$>10^3$
192	--	$>10^3$
216	--	11.2
246	--	116

-- Not counted.
Visible growth in Hz after 246 + hours.

Table 2. Effective concentration of hydrazine fuels to various aquatic species.

FUEL	TEST SPECIES	EC50 ppm	NO EFFECT LEVEL	REFERENCE
Hz	Goldfish (Carassius auratus)	3.7	-----	Hoover et al., 1964
	Catfish (Icalurus punctatus)	2.4	-----	Hoover et al., 1964
	Large Mouth Bass (Micropterus salmoides)	2.7	-----	Hoover et al., 1964
	Algae (Selenastrum capricornutum)	1.2	-----	Hoover et al., 1964
	Microcrustacean (Daphnia pulex)	0.03	.001	Scherfig et al., 1978
	Toad (Xenopus laevis)	12.5	-----	Greenhouse, 1976b
	Stickleback (Gasterosteus aculaetus)	3.4	>3.2 <5.6[2]	Klein and Jenkins, 1978
UDMH	Goldfish (Carassius auratus)	7.0	-----	Hoover et al., 1964
	Catfish (Icalurus punctatus)	4.8	-----	Hoover et al., 1964
	Bass (Micropterus salmoides)	9.0	-----	Hoover et al., 1964
	Algae (Selenastrum capricornutum)	5.0	2.0	Scherfig et al., 1978
	Toad (Xenopus laevis)	9.0	-----	Greenhouse, 1976b
	Stickleback (Gasterosteus aculaetus)	1.6	>3.2 ≈10[2]	Klein and Jenkins, 1978
MMH	Algae (Selenastrum capricornutum)	0.5	0.2	Scherfig et al., 1978
	Toad (Xenopus laevis)	>5 <7.5	-----	Greenhouse, 1976b
	Stickleback (Gasterosteus aculaetus)	0.36[1]	>1.0 <3.2[2]	Klein and Jenkins, 1978

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